

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising
  - (a) a first class switch region ( $S_1$ ) nucleotide sequence of an upstream immunoglobulin locus under transcriptional control of a first promoter;
  - 5 (b) a second class switch region ( $S_2$ ) nucleotide sequence of an immunoglobulin locus downstream of said upstream Ig locus under transcriptional control of a second promoter, wherein said  $S_2$  sequence serves as a region-specific substrate for class switch recombination (CSR);
  - (c) a reporter gene nucleotide sequence encoding a reporter molecule, interposed  
10 between said  $S_1$  and  $S_2$  sequences in reverse transcriptional orientation, and
  - (d) a promoter, downstream of said nucleotide sequence encoding said reporter molecule, allowing the expression of said reporter molecule only following CSR between said  $S_1$  and  $S_2$  sequences.
2. The nucleic acid molecule of claim 1 wherein said  $S_1$  is an  $S_{\mu}$  sequence and  
15 said  $S_2$  is an  $S_{\gamma 2}$  sequence.
3. The nucleic acid molecule of claim 1 wherein said  $S_1$  is an  $S_{\mu}$  sequence and said  $S_2$  is an  $S_{\epsilon}$  sequence.
4. The nucleic acid molecule of claim 2 wherein said  $S_1$  and  $S_2$  sequences are G-rich switch region DNA sequences.
- 20 5. The nucleic acid molecule of claim 3 wherein said  $S_1$  and  $S_2$  sequences are G-rich switch region DNA sequences.
6. The nucleic acid molecule of claim 1 wherein said nucleic acid in part (c) and said promoter in part (d) are under control of an internal ribosome entry site (IRES).
7. The nucleic acid molecule of claim 1 wherein said nucleic acid in part (c)  
25 encodes a Green Fluorescent Protein (GFP) molecule.
8. The nucleic acid molecule of claim 1 wherein said nucleic acid in part (c) encodes a reporter molecule selected from the group consisting of  $\beta$ -galactosidase, luciferase, and secreted alkaline phosphatase (SEAP).
9. The nucleic acid molecule of claim 1 wherein said first and second  
30 promoters are non-inducible constitutive promoters.

10. The nucleic acid molecule of claim 9 wherein said first promoter is a CMV promoter.

11. The nucleic acid molecule of claim 9 wherein said second promoter is an SV promoter.

5           12. An isolated nucleic acid molecule comprising  
          (a) a human S $\mu$  nucleotide sequence under control of a CMV promoter;  
          (b) a human S $\gamma_2$  nucleotide sequence under control of an SV promoter;  
          (c) an RSV LTR enhancer/promoter and GFP gene under control of an internal  
              ribosome entry site (IRES), interposed between said S $\mu$  and S $\gamma_2$  sequences,  
10           in reverse transcriptional orientation,  
          (d) a 5' splicing donor site from human  $\beta$ -globulin gene, 3' of said S $\mu$  sequence;  
              and  
          (e) a 3' splicing acceptor site and C $\epsilon$ 1 exon, 3' of said S $\gamma_2$  sequence.

15           13. The nucleic acid molecule of claim 12 further comprising a nucleic acid  
          fragment of a cytokine-inducible promoter for Ig germline transcription, 5' of said  
          CMV promoter.

          14. The nucleic acid molecule of claim 13 wherein said cytokine-inducible  
          promoter is an IL-4 inducible I $\epsilon$  promoter.

20           15. The nucleic acid molecule of claim 12 selected from the group consisting of  
          XF-1, XF-5a, XF-8, XF-2a, XF-2b, XF-6a and XF-6b.

          16. A switch vector comprising a nucleic acid molecule of claim 1.

          17. A switch vector comprising a nucleic acid molecule of 12.

          18. A recombinant host cell stably transfected with the switch vector of claim  
16.

25           19. A recombinant host cell stably transfected with the switch vector of claim  
17.

          20. The host cell of claim 18 which is a mammalian cell.

          21. The host cell of claim 20, which is a Chinese Hamster Ovary (CHO) cell.

          22. The host cell of claim 20 which is a primary human B cell.

30           23. A method of monitoring immunoglobulin (Ig) class switch recombination  
          (CSR), comprising

(a) providing a switch vector comprising

- (i) a first class switch region ( $S_1$ ) nucleotide sequence of an upstream Ig locus under transcriptional control of a first promoter;
- (ii) a second class switch region ( $S_2$ ) nucleotide sequence of an Ig locus downstream of said upstream Ig locus under transcriptional control of a second promoter, wherein said  $S_2$  sequence serves as a region-specific substrate for CSR;
- (iii) a reporter gene nucleotide sequence encoding a reporter molecule interposed between said  $S_1$  and  $S_2$  sequences in reverse transcriptional orientation, and
- (iv) a promoter, downstream of said nucleotide sequence encoding said reporter molecule, allowing the expression of said reporter molecule only following switch recombination between said  $S_1$  and  $S_2$  sequences;

(b) stably transfecting a mammalian cell with said switch vector; and

(c) monitoring the expression of said reporter molecule in said mammalian cell, wherein such expression indicates CSR.

24. The method of claim 23 wherein said mammalian cell is a primary B cell or a B cell line.

25. The method of claim 24 wherein said B cell line is a human B lymphoma cell line.

26. The method of claim 25 wherein said cell line contains a single copy of said switch vector.

27. The method of claim 23 wherein said reporter molecule is Green Fluorescent Protein (GFP).

28. The method of claim 27 wherein CSR is monitored by fluorescence microscopy.

29. The method of claim 28 further comprising the step of quantifying CSR.

30. The method of claim 29 wherein said CSR is quantified by flow cytometry.

31. The method of claim 29 wherein said first promoter is a CMV promoter.

32. The method of claim 29 wherein said second promoter is an SV promoter.

33. The method of claim 31 wherein said switch vector further comprises a cytokine-inducible promoter for Ig germline transcription 5' of said CMV promoter.

34. The method of claim 33 wherein said cytokine-inducible promoter is an IL-4 inducible I $\epsilon$  promoter.

5           35. The method of claim 34 further comprising the step of culturing said cells in the presence of IL-4 and/or a stimulator of CD40 activity prior to monitoring CSR.

36. The method of claim 35 wherein said stimulator of CD40 activity is an anti-CD40 monoclonal antibody (mAb) or a CD40 ligand.

37. The method of claim 35 further comprising the step of exposing said cells to  
10 a candidate molecule, and determining the effect of said candidate molecule on GFP expression.

38. A method of monitoring immunoglobulin (Ig) class switch recombination (CSR) comprising

- 15           (a) providing a switch vector comprising, under transcriptional control of a promoter and in natural transcriptional orientation,
- (i) a first class switch region ( $S_1$ ) nucleotide sequence of an upstream Ig locus;
- (iii) a second class switch region ( $S_2$ ) nucleotide sequence of an Ig locus downstream of said upstream Ig locus; and
- 20           (iv) a reporter gene nucleotide sequence encoding a reporter molecule, interposed between said  $S_1$  and  $S_2$  sequences;
- (b) incubating said switch vector with a cell-free nuclear extract from Ig-producing cells or cells with Ig-producing potential; and
- (c) detecting deletion of said reporter gene.

25           39. The method of claim 38 wherein said first class switch region sequence ( $S_1$ ) is an  $S_{\mu}$  sequence and said second class switch region sequence ( $S_2$ ) is an  $S_{\epsilon}$  sequence.

40. The method of claim 38 wherein deletion of said reporter gene is detected following transformation of said switch vector into a recombinant host cell.

30           41. The method of claim 40 wherein said recombinant host cell is a prokaryotic cell.

42. The method of claim 41 wherein said prokaryotic cell is an *E. coli* cell.
43. The method of claim 40 wherein said reporter gene is a lacZ gene.
44. The method of claim 43 wherein deletion of said reporter gene is detected by counting the white colonies obtained after transformation, in the presence of isopropyl- $\beta$ -D-thiogalactoside (IPTG) and 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactoside (X-gal).
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45. The method of claim 38 wherein said Ig-producing cells are B lymphocytes.
46. The method of claim 45 wherein said B lymphocytes are of human origin.
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47. The method of claim 38 wherein said Ig-producing cells are primary B cells stimulated with CD40.
48. The method of claim 38 wherein said S<sub>1</sub> and S<sub>2</sub> comprise G-rich, tandemly repetitive sequences.